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Chapter 21

MEMBRANE DELIVERED ETHENE TO STIMULATE MICROBIAL DEGRADATION OF DCE

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ABSTRACT

A significant obstacle to the application of microbial reductive dechlorination of PCE and TCE is the undesirable accumulation of *cis*-DCE and vinyl chloride. In this study, lab-scale aquifer sediment column experiments were conducted to evaluate the feasibility of using ethene to stimulate cometabolic and/or auxiliary aerobic degradation of DCE in aquifer sediments from Edwards Air Force Base (EAFB). A mixture of ethene in air was supplied to gas-permeable membranes installed in a test column to stimulate aerobic degradation of DCE by ethenotrophic populations. Membranes in a parallel column were supplied with air or N_2 as a negative control. The experimental results indicated that simply supplying ethene and air to the EAFB aquifer sediments alone did not produce conditions favorable for growth of DCE-degrading ethenotrophs. Moreover, amending the aquifer sediments with nutrients and bioaugmenting with enriched and pure (*Nocardioides* strain JS614) ethenotrophic cultures failed to stimulate growth of DCE-degrading ethenotrophs. This may have been due to the presence of inhibitory substrates or the absence of requisite growth factors. Parallel microcosm studies demonstrated that both the enriched ethenotrophic culture and the *Nocardioides* strain JS614 culture rapidly cometabolized DCE in mineral salts media, but did not readily acclimate to the EAFB aquifer sediments.

Keywords: bioaugmentation bioremediation, dichloroethene, cometabolism, ethene, membrane

1. INTRODUCTION

1.1 Research Objective

Cis-dichloroethene (*cis*-DCE) and vinyl chloride (VC) are formed during anaerobic reductive dechlorination of perchloroethene (PCE) and trichloroethene (TCE). Although complete dechlorination to ethene *in situ* has been demonstrated, persistence of *cis*-DCE and VC under anaerobic conditions has impeded success of both natural attenuation and enhanced reductive

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dechlorination at a large number of sites. One explanation is that *cis*-DCE/VC dehalorespirers are often absent from contaminated sites (Fennell *et al.*, 2001). Another potential explanation is that *cis*-DCE/VC dehalorespirers are subject to substrate-limited growth; for example, Cupples *et al*. (2004) concluded that dehalorespirers theoretically should be unable to grow on VC at concentrations below ~44 μg/L. As an alternative to reductive dechlorination, many studies have demonstrated that *cis*-DCE and VC can be readily degraded under aerobic conditions (Coleman 2002a and references therein). Degradation of *cis*-DCE and VC under aerobic conditions, however, may also be impeded by absence of the requisite microbial populations and/or substrate-limited growth conditions. In addition, *cis*-DCE and VC typically coexist with PCE and TCE in groundwater plumes (Suarez *et al.*, 2004), and thus supplying oxygen to promote aerobic conditions would inhibit reductive dechlorination of PCE and TCE. These obstacles could be circumvented by developing a bioremediation technology involving (*i*) bioaugmentation with an aerobic microbial population capable of growing on *cis*-DCE and VC, (*ii*) supply of an auxiliary growth substrate capable of sustaining growth of the bioaugmented population without inhibiting secondary metabolism of *cis*-DCE and VC, and (*iii*) supply of oxygen without inhibiting anaerobic PCE/TCE dechlorination. Towards developing such a technology, this study assessed the feasibility of stimulating the growth of DCE-degrading ethenotrophic microbial populations in a lab-scale column packed with EAFB aquifer sediments and supplied with a 6% ethene in air mixture using gas-permeable hollow-fiber membranes. Parallel microcosm studies were also performed to assess the feasibility of stimulating growth of DCE-degrading ethenotrophs in the EAFB aquifer sediments (Clapp et al., 2008).

1.2 Factors Impacting Feasibility of Using Membrane-delivered Ethene to Stimulate Growth of Ethenotrophs for Secondary *cis*-DCE Biodegradation

At many sites bioaugmentation may be required to promote growth of aerobic DCE/VC-oxidizing bacteria in the aquifer. Several studies have verified the existence of bacteria that can grow by oxidizing VC as the sole carbon source (Hartmans and de Bont, 1985; Verce et al., 2000, 2001, 2002). Coleman et al. (2002a) conducted a study to assess the diversity and distribution of aerobic bacteria that grow on VC and detected VC degradation in 23 of 37 samples collected from 22 sites, and furthermore isolated 11 Mycobacterium strains and 1 Nocardioides strain. Thus, bacteria capable of growing on VC appear to be common in the environment, but not ubiquitous. Coleman et al. (2002b) also recently conducted a study to assess the existence of aerobic bacteria that grow on cis-DCE and obtained 2 active cultures from 18 enrichments, and furthermore isolated one aerobic β-proteobacterium strain that can grow on cis-DCE as a sole carbon and energy source. They conducted a study to assess the existence of aerobic bacteria that grow on cis-DCE as a sole carbon and energy source and concluded that these organisms are rare in the environment and may only exist in highly selective environments.

Successful bioaugmentation may be impeded by inefficient transport and dispersal of injected bacteria in the subsurface. Microbial transport in the subsurface depends on the characteristics of both the cells and the surrounding porous media (Ginn et al., 2002). Several field studies with forced gradient groundwater flow found that injected bacteria do not travel far from the injection point (ESTCP, 2005). In general, observed advective transport of less than 3 m appears to be common for bacteria in silt or fine sand deposits (Harvey, 1997).

VC and cis-DCE may only support net growth of aerobic bacteria when present at concentrations well above MCL values. Microbial growth rate is a function of substrate concentration. At sufficiently low concentration (S_{min}) the decay rate exceeds the growth rate. Bradley and Chapelle (2000) found that bacteria indigenous to stream sediments could utilize cis-DCE as a sole carbon substrate for aerobic metabolism, but could not grow at concentrations below 4850 µg/L, and Rittman and Brunner (1984) have reported that aerobic bacteria cannot sustain net growth on simple compounds at concentrations below 100 to 1000 µg/L.

Ethene can serve as an auxiliary substrate to support secondary aerobic metabolism of cis-DCE and VC. Aerobic ethene-utilizing bacteria can readily cometabolize cis-DCE (Koziollek et al., 1999), and known VC-assimilating bacteria readily grow on ethene (Coleman et al., 2002a). In aerobic VC assimilation, the first step involves monooxygenase-catalyzed reactions that produce VC epoxide (Verce et al., 2000). Chloroethene epoxides are chemically unstable and rapidly degrade to a variety of products that are toxic to cells (Fox, 1990), which accounts for the low VC transformation yields attained through cometabolic degradation by methanotrophs and other oxygenase-expressing bacteria (Dolan and McCarty, 1995). Unlike all other substrates that have been used to support aerobic degradation of VC and cis-DCE via cometabolism, ethene can serve as a co-substrate for growth-coupled secondary metabolism of VC (Fogel et al., 2005). Coleman et al. (2003) recently found that Mycobacterium strains isolated on either ethene or VC possessed epoxyalkane:coenzyme M transferase (EaCoMT) enzymes that channel the generated epoxides into metabolic pathways and thus guard against accumulation of the toxic epoxides in the cytoplasm. This probably explains why cis-DCE transformation yields (i.e., moles of cis-DCE mineralized per mole of substrate utilized) attained with ethene as an auxiliary growth substrate are an order of magnitude greater than when methane is used (Koziollek et al., 1999).

Ethenotrophic degradation of VC and cis-DCE is inhibited by high ethene concentrations. As described above, ethene possesses advantages over other substrates (e.g., methane). However, as is the case for the other substrates, ethene will compete for monooxygenase active sites and thus competitively inhibit VC and cis-DCE degradation.

Gas-permeable membranes can be used to supply dissolved gases to the subsurface with very high transfer efficiency. The iSOC® membrane technology has recently been used to supply ethene and oxygen to a VC-contaminated aquifer (Fogel et al., 2005; LeBlanc et al., 2005). Gas-permeable hollow-fiber membranes have also been used for in situ hydrogen transfer to stimulate reductive dechlorination (Edstrom et al., 2005; Clapp et al., 2004a; Ma et al., 2003; Fang et al., 2002). The use of gas-permeable membranes has the advantage of preventing gas bubble formation, and thus avoiding potential accumulation of explosive gas mixtures in the vadose zone or well head.

It may be possible to concentrate VC/DCE-degrading ethenotrophs on gas-permeable membranes installed within groundwater circulation wells. Previous studies (Clapp et al., 2004b; Sumani et al., 2005) have demonstrated that robust methanotrophic biofilms will rapidly grow on gas-permeable membranes supplied with CH_4 and air. These studies also indicated that high CH_4 concentrations and low TCE concentrations at the biofilm interior resulted in optimal methanotrophic growth conditions, while low CH_4 and high TCE concentrations near the biofilm exterior minimized competitive inhibition. These results suggest that bioaugmented cis-DCE and

VC degrading ethenotrophs could similarly be concentrated on gas-permeable hollow-fiber membranes**.**

By combining the in-situ membrane approach with groundwater circulation well (GCW) technology, the number of wells required to effectively treat the width of a plume can be minimized. In GCWs, groundwater is extracted from one screened interval and discharged through a second screened interval at a different level, creating a groundwater circulation pattern around the wells. If the GCWs are equipped with membrane-attached ethenotrophic biofilms, they will effectively become in-situ bioreactors (Veerasekaran, 2004).

2. MATERIALS AND PROCEDURE

2.1 Aquifer Sediment Column Studies

Aquifer sediment columns were used to assess the ability of membrane-delivered ethene and air to stimulate degradation of *cis*-DCE by ethenotrophic bacteria indigenous to EAFB aquifer sediments. The same aquifer sediment columns were used to determine if nutrient amendment and bioaugmentation with an enriched ethenotrophic culture would improve *cis*-DCE degradation in the EAFB aquifer sediments. Two aquifer sediment columns were fabricated (Figure 1a). Each column consisted of three 9.2-cm i.d. × 31 cm glass sections, with two 3.5-cm thick polycarbonate membrane modules (Figure 1b) placed in between the sections. To allow collection of aqueous samples, one sampling port in the bottom glass sections, three in the middle and top sections, and one in each of the membrane modules were installed. Each membrane module contained two lengths of gas-permeable Varglas ES-4400 (Varflex Corp., Rome, NY) silicone-coated fiberglass membrane tubing threaded in parallel through aluminum cross supports in a spiral configuration as shown in Figure 1b to provide a uniform ethene and oxygen supply across the column cross sections. The bottom column sections were filled with 1-mm glass beads to provide uniform hydraulic flow. Two aquifer sediment columns were wet-packed with the homogenized aquifer sediments collected from the EAFB site. Tubing was connected to the inlet and outlet of columns. The outlet lines delivered the effluent to collection flasks. The inlet lines were connected to a 50-L carboy that served as a groundwater feed reservoir. Two piston pumps were used to deliver the groundwater to the columns. A tee was installed in each inlet line and connected to a multi-channel syringe pump that was used to spike the groundwater with a concentrated *cis*-DCE solution. Approximately 30 gallons of aquifer sediments were collected from EAFB borehole Number 284-MW03 for packing the column reactors and providing sediment slurry for the microcosm experiments. A mixture of 6% ethene in air (for the test column) and either air or nitrogen (for the control column) were supplied to the membrane modules from gas cylinders. An infrared sensor (Detcon, Inc.) was connected to the outlet gas line of the test column so that continuous ethene delivery could be monitored.

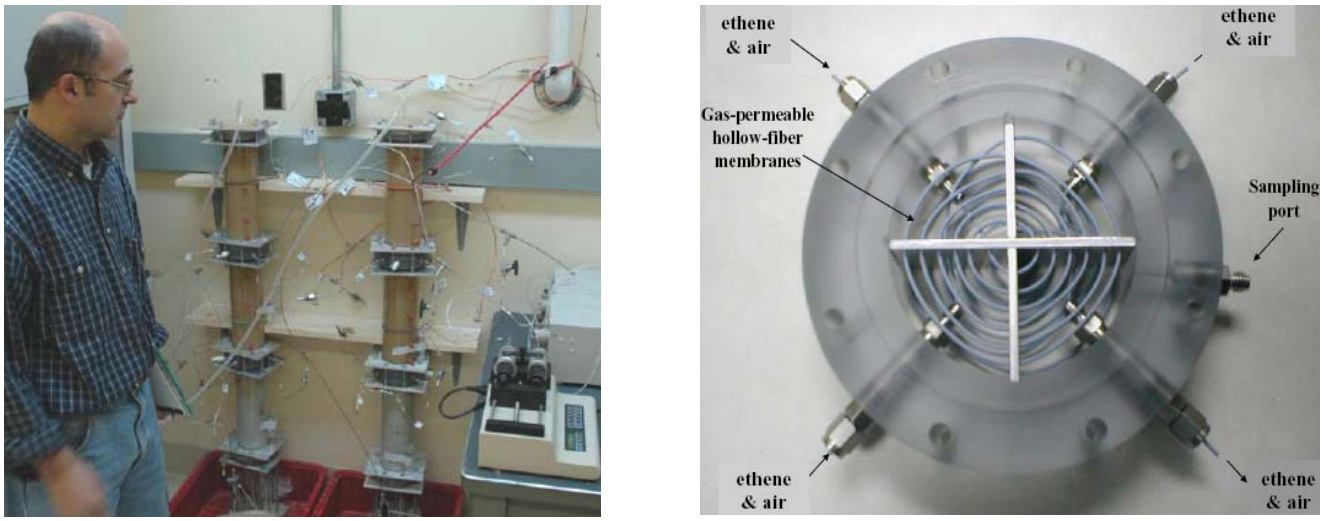

Figure 1. (a) Aquifer sediment column reactors with piston pumps and syringe pump on the right; (b) membrane modules, each with two 100-cm hollow-fiber silicone membranes.

Table 1. Operating conditions for aquifer sediment columns C1 and C2.[1]

		Target influent DCE conc. (µg/L)		Gas supplied [2]		Gas flow per membrane (mL/min)		Nutrient addition		Ethenotroph bioaugmentation	
Study	**Start**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**
A) Br^- tracer	01/26/07	0	0	air	air	3	3	No	No	No	No
B) DCE retardation	03/12/07	50	50	air	air	3	3	No	No	No	No
C) Ethene turned on for C1	04/03/07	500	500	eth/air	air	3	3	No	No	No	No
D) Lowered gas flows	05/24/07	500	500	eth/air	air	1	1	No	No	No	No
E) Switched to N_2 in C2	07/28/07	500	500	eth/air	N_2	1	1	No	No	No	No
F) Added culture & nutrients	10/19/07	500	500	eth/air	N_2	1	1	Yes	Yes	Yes	No
G) Turned off gas to C2	11/15/07	500	500	eth/air	none	1	0	Yes	Yes	No	No

[1] The groundwater flow rate through both aquifer sediment columns was maintained at 0.5 mL/min (or 0.72 L/day) throughout all the studies, corresponding to a linear velocity of ~31 cm/day.

[2] A mixture of 6% ethene in air was supplied to column C1 during studies C-G.

An overview of the column operating parameters for the *cis*-DCE degradation studies is presented in Table 1. The aquifer sediment column *cis*-DCE degradation studies were comprised of seven phases (A-G), as described in the data and analysis section.

2.2 Enriched Culture and Aquifer Sediment Microcosm Studies

To complement the aquifer sediment column studies, enriched ethenotrophic culture studies were performed (Figure 2a) to verify that ethenotrophic cultures could rapidly degrade *cis*-DCE with high transformation yields. In addition, EAFB aquifer sediment microcosm studies (Figure 2b) were conducted to determine (1) if growth of indigenous ethenotrophic bacteria present in the EAFB aquifer sediments could be stimulated in the presence of ethene and oxygen, (2) if nutrient (N and P) availability in the EAFB aquifer sediments limited growth of ethenotrophic bacteria, and (3) if bioaugmentation with an enriched ethenotrophic culture would stimulate growth of ethenotrophs in the EAFB aquifer sediments (Bandyopadhyay, 2008).

Figure 2. (a) Subcultures of enriched ethenotrophic cultures on a platform shaker; (b) sediment microcosm bottles being analyzed for ethene and CO_2.

3. DATA AND ANALYSIS

3.1 Results of Aquifer Sediment Column Studies

3.1.1 Conservative tracer (study A)

The hydraulic characteristics of the columns were evaluated using a tracer study, with the columns operated in an up-flow mode. To simulate groundwater conditions, groundwater was pumped through the two columns at 0.5 mL/min. The groundwater feed to one of the columns was spiked with 60 mg/L of bromide, and 5-mL water samples were collected from the last port once a day for 16 days and analyzed for Br^- breakthrough using ion chromatography (Figure 3). The column cross-sectional area was $A_c = 67$ cm^2, and thus the specific discharge was $v = Q/A_c = 10.7$ cm/day. Since the porosity of the membrane modules was 1.0, this was also the

groundwater linear velocity through the membrane modules. Bromide at the top sampling port reached 30 mg/L (50% of the inlet concentration) at approximately $\theta = 4$ days after initiating the bromide spiking. Since total distance from the bottom of the column to the top sampling port was L = 123 cm, the average linear groundwater velocity through the column was $v_{lin} = L/\theta = 31$ cm/day. The effective porosity of the porous media was calculated as $\eta = v/v_{lin} = 0.34$.

3.1.2 *Cis*-DCE breakthrough curve (study B)

After the tracer study, a *cis*-DCE breakthrough study was performed. The influent line to each column was spiked with a concentrated *cis*-DCE solution at 5 µL/hr using a syringe pump. To characterize the *cis*-DCE breakthrough curves, 1-mL water samples were withdrawn from the last port of each column every day over two weeks and analyzed for *cis*-DCE using an equilibrium headspace method with a gas chromatograph equipped with an electron capture detector (GC-ECD). Figure 3 compares the breakthrough curves for bromide and *cis*-DCE. The *cis*-DCE concentration at the top sampling port reached 25 µg/L (50% of the inlet concentration) at approximately $\theta = 15$ days after initiating the *cis*-DCE spiking. Thus, the *cis*-DCE took roughly 3.7 times as long to break through the columns, implying a low retardation factor of 3.7, which was consistent with the low organic carbon content of the sediments.

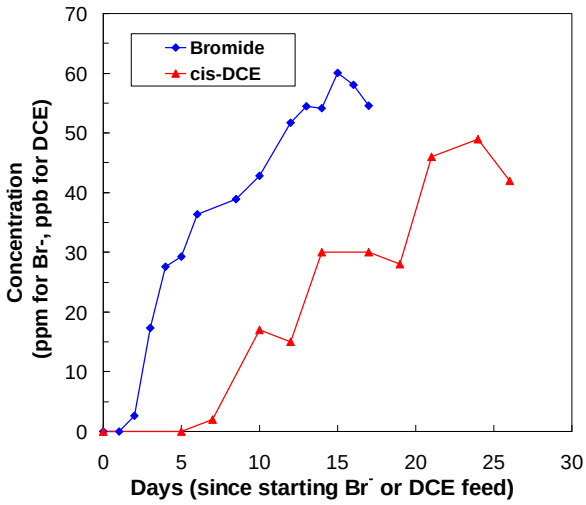

Figure 3. Bromide and cis-DCE breakthrough data for aquifer sediment column.

3.1.3 Baseline *cis*-DCE removal (study C)

Following the bromide tracer and *cis*-DCE breakthrough studies, a baseline *cis*-DCE degradation study was conducted to assess whether supply of ethene and air through the gas-permeable membranes would stimulate growth of indigenous ethenotrophic bacteria in the EAFB aquifer sediments. Ethene supply (6% in air by volume) to column C1 was initiated, while column C2 continued to be supplied with air alone as a negative control. The gas flow rate through each hollow-fiber membrane was maintained at ~3 mL/min. The *cis*-DCE concentrations at the bottom and top sampling ports for the ethene-supplied test column (C1) and air-supplied

control column (C2) are shown in Figure 4. The average *cis*-DCE removals in the ethene-supplied test column (C1) and air-supplied control column (C2) during the study C were 83 ± 13% and 78 ± 14%, respectively (with the ± values indicating 90% confidence intervals).

3.1.4 *cis*-DCE removal after lowering gas flows (study D)

Because high *cis*-DCE removals were observed in both the ethene-supplied test column and the air-supplied control column during study C, it was hypothesized that pervaporation of *cis*-DCE through the hollow-fiber membranes was occurring. Therefore, the gas flows were decreased from ~3 mL/min per hollow fiber membrane to ~1 mL/min per fiber (or, since there were four fibers per column, from a total of ~12 mL/min per column to ~4 mL/min per column). This change in operating conditions resulted in the average *cis*-DCE removals in columns C1 and C2 decreasing from 83 ± 13% and 78 ± 14% to 47 ± 14% and 47 ± 19%, respectively (Figure 4), which supported the pervaporation hypothesis.

3.1.5 *cis*-DCE removal after switching from air to N_2 in control column (study E)

Because relatively high *cis*-DCE removals were observed in both the ethene- and air-supplied columns during study D, even at the low gas flow rates, it was hypothesized that supply of air was stimulating aerobic biodegradation of *cis*-DCE in the control column (C2). Therefore, air was replaced with N_2 for the gas supply to column C2. However, *cis*-DCE removal in the control column remained comparable to that in the test column (66 ± 26% and 61 ± 18%, respectively; see Figure 4), which was counter to the air supply hypothesis. This suggested that continued high *cis*-DCE removal in the control column was not due to aerobic degradation, although the possibility that dissolved oxygen in the feed groundwater was supporting aerobic degradation could not be ruled out.

3.1.6 *cis*-DCE removal after bioaugmentation and nutrient amendment (study F)

Because the *cis*-DCE removal in ethene-supplied test column was not greater than in the N_2-supplied control column after six months of continuous operation, it was hypothesized that either lack of nutrients and/or absence of indigenous microbes was inhibiting growth of *cis*-DCE degrading ethenotrophs in the test column. Therefore, the feed groundwater for both columns was amended with 10 mg/L of NO_3-N and 2 mg/L of PO_4-P, and the test column was bioaugmented with two enriched DCE-degrading cultures. A DCE-degrading mixed ethenotrophic culture was obtained from Bioremediation Consultants Inc. (Watertown, MA) and a pure culture of *Nocardioides* strain JS614 was obtained from University of Iowa. Both cultures were grown to concentrations of approximately 500 mg VSS/L in serum bottles containing 40 mL of a mineral salts medium (MSM) and supplied with a 6% ethene in air headspace (Figure 2a). To bioaugment the test column (C1), 10 mL of each culture was injected into both the bottom and top membrane module sampling ports. However, even after bioaugmenting the test column, the *cis*-DCE removals in the two columns remained comparable (Figure 4).

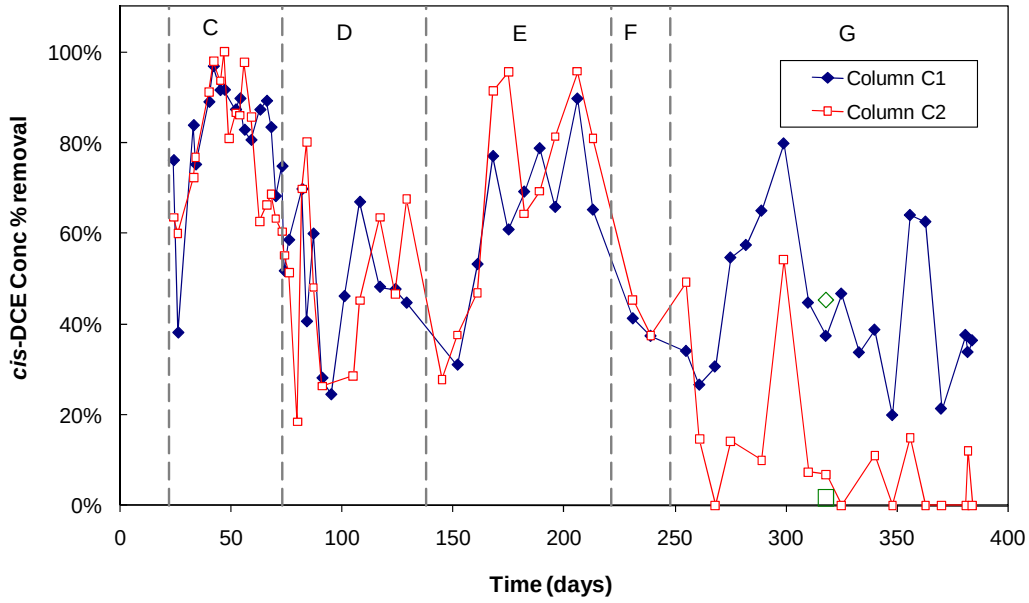

Figure 4. *cis*-DCE removals in the ethene-supplied test column (C1) and control column (C2) over time. The operating conditions for each period are summarized in Table 1. The open symbols on day 318 were for split sample analyses conducted at a second laboratory.

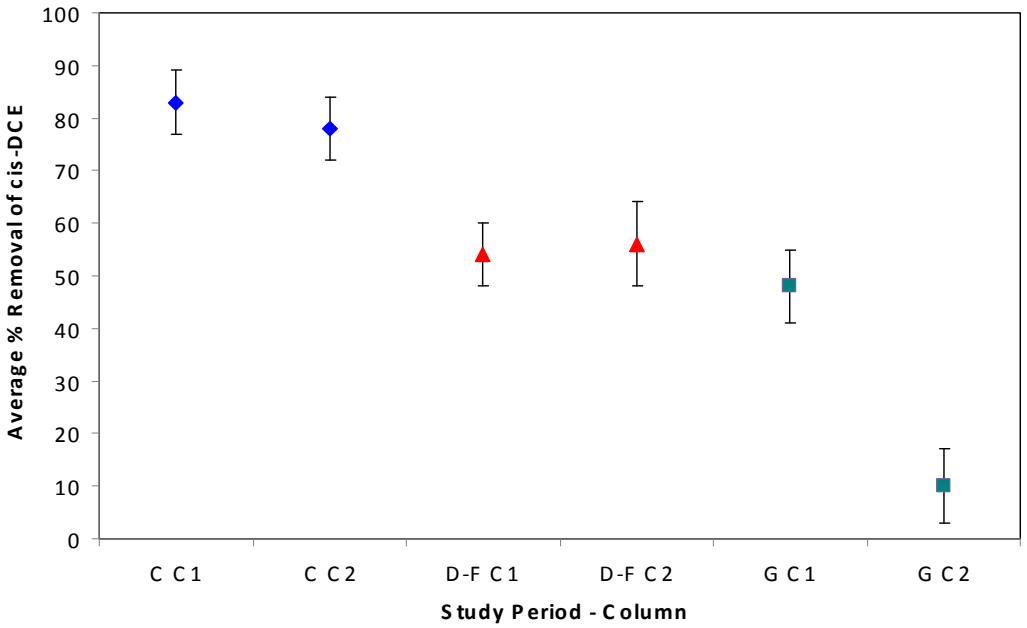

Figure 5. Average *cis*-DCE removal efficiencies in the test column (C1) and control column (C2) during the three different gas flow periods. Error bars represent 90% confidence intervals. The first letters on the *x*-axis indicate the study period (C, D-F, or G).

3.1.7 *cis*-DCE removal after turning off gas supply to control column (study G)

The *cis*-DCE removals in the ethene-supplied test column (C1) did not become significantly greater than in the N_2-supplied control column (C2) after 247 days of *cis*-DCE spiking, even after bioaugumenting the test column. Therefore, it was hypothesized that the *cis*-DCE removal observed in both columns was still primarily due to pervaporation, as opposed to biodegradation. To test this hypothesis, all gas flow to the control column was stopped. There was no significant change in the *cis*-DCE removal in the ethene-supplied test column, indicating that the nutrient addition and bioaugmentation had not improved the *cis*-DCE removal rates. In contrast, the average *cis*-DCE removal in the control column decreased from 66 ± 26% during study E to 5 ± 17% during study G (Figure 4), indicating that, as hypothesized, pervaporation was probably the primary *cis*-DCE removal mechanism in both columns.

3.1.8 Comparison of *cis*-DCE removal under different gas flow conditions (studies C-G)

To characterize the effect of gas flow rate, Figure 5 compares the *cis*-DCE removals in both columns under the three different gas flow conditions used. It is evident that the time-averaged *cis*-DCE removal rates decreased significantly when the gas flow rate was decreased from ~3 mL/min/fiber to ~1 mL/min/fiber (for both columns), and again when the gas flow rate was decreased from ~1 mL/min/fiber to zero (for column C2 only). In contrast, there was no statistically significant difference between the *cis*-DCE removals in the ethene-supplied test column (C1) and the air- or N_2-supplied control column (C2) when both were supplied with gas at the same flow rates. These results were all consistent with the *cis*-DCE removal in both columns resulting primarily from pervaporation through the hollow-fiber membranes, and not from biodegradation. This interpretation of the data was supported by average *cis*-DCE concentration profiles for both columns that consistently showed decreases in *cis*-DCE concentrations between the sampling ports immediately upstream and downstream of the two membrane modules (Clapp et al., 2008). As will be discussed in section 4.2, this interpretation of the data was also consistent with the results of microcosm that showed little stimulation of ethenotrophic activity, even after bioaugmentation.

3.1.9 Dissolved ethene concentrations in test column

During study G, liquid samples were collected from sampling ports in the bottom and top membrane modules of the test column (C1). In addition, the gas outlet line from the test column was placed in a 20-mL glass vial filled with DI water, such that the gas bubbled into the water. The collected samples (with zero headspace) were analyzed for ethene using a GC equipped with a thermal conductivity detector (TCD). The DI water that had been bubbled with the outlet gas had an ethene concentration of 6.40 ± 0.28 mg/L. In comparison, using the Henry's law constant for ethene at 25°C of 213 atm/M (Sander, 1999), the theoretical ethene concentration for water at equilibrium with ethene gas at a partial pressure of 0.06 atm was calculated to be 7.89 mg/L. Thus, the ethene concentration in the water that had been bubbled with the outlet gas from the membrane modules was only slightly lower than the theoretical saturation concentration assuming an ethene partial pressure of 0.06 atm, indicating that the ethene composition of the gas flowing through the membranes was close to the target value of 6%.

The dissolved ethene concentrations in the water samples collected from the bottom and top membrane module sampling ports were similar at 0.83 ± 0.28 and 0.87 ± 0.09 mg/L, respectively. In comparison, a dimensionless membrane mass transfer correlation developed by Fang et al. (2002) was used to predict that the dissolved ethene concentrations under abiotic conditions would have been ~2.52 mg/L. Thus, the actual measured dissolved ethene concentrations, 0.83 and 0.87 mg/L, were only 33% and 35%, respectively, of the concentration that theoretically should have existed under abiotic conditions. This experimental finding was consistent with the possibility that ethenotrophic bacteria were utilizing ethene within the test column.

3.1.10 Presence of biofilms on membranes in the test column

At the conclusion of the experiment, the columns were disassembled and the membrane modules visually inspected. Figure 6 shows that significantly more robust biofilms developed on the membranes in the test column than on the membranes in the control column, indicating more microbial activity on the test columns. Evidence of minor mineral scaling (e.g., $CaCO_3$) was also observed on the membranes from both columns.

Figure 6. Biofilms on the bottom membranes in the control column (CB) and test column (TB).

3.1.11 Alkalinity and pH data

The effluent alkalinity and pH for the two columns were measured weekly. During studies C-E, the effluent alkalinities ranged from 90 to 120 mg/L (as $CaCO_3$) in both columns. However, the effluent alkalinities in both columns increased to between 120 and 160 mg/L as $CaCO_3$ during studies F-G when the feed groundwater was spiked with 10 mg/L of NO_3-N and 2 mg/L of PO_4-P. The increase in alkalinity may have been due to the nitrate addition, since denitrification of 10 mg/L of NO_3-N would theoretically increase alkalinity by 35 mg/L as $CaCO_3$ (Metcalf & Eddy Inc. et al., 2002). Despite periodic additions of HCl to the groundwater feed to lower the influent pH, the effluent pH values for the control column (C2) were consistently between 8.7 and 9.1. The effluent pH values for the ethene-supplied test column (C1) also fell within this range during studies C and D. However, during studies E-G, the test column effluent pH generally decreased to between 8.2 and 8.8, or 0.5 units lower, on average, than for the control column. This result was consistent with the possibility that ethenotrophic

bacteria were active in the ethene-supplied test column, since ethene oxidation to CO_2 would decrease the effluent pH.

3.2 Results of Enriched Culture and Aquifer Sediment Microcosm Studies

3.2.1 Verification of *cis*-DCE degradation by enriched ethenotrophic cultures

As described above, both a mixed ethenotrophic culture and a *Nocardioides* strain JS614 pure culture were grown in serum bottles (Figure 2a). Observed growth yields, Y_{obs}, were 1.62 ± 0.05 and 1.89 ± 0.35 mg of VSS per mg of ethene, respectively. The Y_{obs} values were determined by measuring initial and final ethene concentrations in the headspace, as well as initial and final VSS concentrations. To assess *cis*-DCE degradation, 160-mL serum bottles containing 40 mL of both ethenotrophic cultures in early stationary growth phase were opened and briefly (< 1 minute) sparged with air to remove residual ethene. The bottles were then recapped with Teflon-coated septa and spiked with 10 ppm *cis*-DCE (based on initial liquid phase volume). Negative controls were similarly prepared by spiking 40 mL of DI water. A dimensionless Henry's constant of 0.167 for *cis*-DCE at 25°C (Sander 1999) was used to compute the aqueous and headspace *cis*-DCE concentrations. The bottles were then placed on a platform shaker at 150 rpm, and the headspace *cis*-DCE concentrations over time were measured by GC-ECD. The first time the cultures were spiked with *cis*-DCE, it took about 6 hrs to remove more than 90% of *cis*-DCE (Figure 7). However, with each respiking the rate of *cis*-DCE transformation decreased, and after the sixth respiking it almost ceased completely, indicating that in the absence of ethene the resting cells had a finite *cis*-DCE transformation capacity, T_c. The T_c values for the mixed ethenotrophic culture and the *Nocardioides* strain JS614 culture were determined to be 0.079 ± 0.020 and 0.083 ± 0.022 mg *cis*-DCE/mg VSS, respectively. These results indicated that neither culture was able to utilize *cis*-DCE as a growth substrate, and that the degradation occurred via cometabolism. The *cis*-DCE transformation yields, T_y, defined as the maximum mass of *cis*-DCE transformed by the resting ethenotrophic cells per unit mass of ethene used for cell growth (Alvarez-Cohen and McCarty, 1991), were determined to be 0.128 ± 0.042 and 0.156 ± 0.015 mg *cis*-DCE/mg ethene, respectively (calculated as $T_y = Y_{obs} \times T_c$). Finally, a peak for a significant transient *cis*-DCE transformation product appeared in the GC-ECD chromatograms that may have been dichloroacetaldehyde, although this was not confirmed.

3.2.2 Evaluation of ethene utilization in non-bioaugmented EAFB aquifer sediments

To evaluate the feasibility of stimulating growth of indigenous ethenotrophic bacteria in the EAFB aquifer, sediment slurry samples (50 mL) were distributed to 12 different 160-mL glass serum bottles, followed by addition of 50 mL of the EAFB groundwater. Three additional serum bottles were filled with 100 mL of DI water to serve as negative controls. The pH of all the microcosm bottles was adjusted to between 7 and 8 using HCl. The 15 serum bottles were then capped with Teflon-lined rubber septa and a gas-tight syringe was used to add ethene to the 60-mL headspaces to yield a ~5% ethene in air mixture. All the serum bottles were then placed on a platform shaker at 150 rpm (Figure 2b), and the headspace ethene and CO_2 concentrations

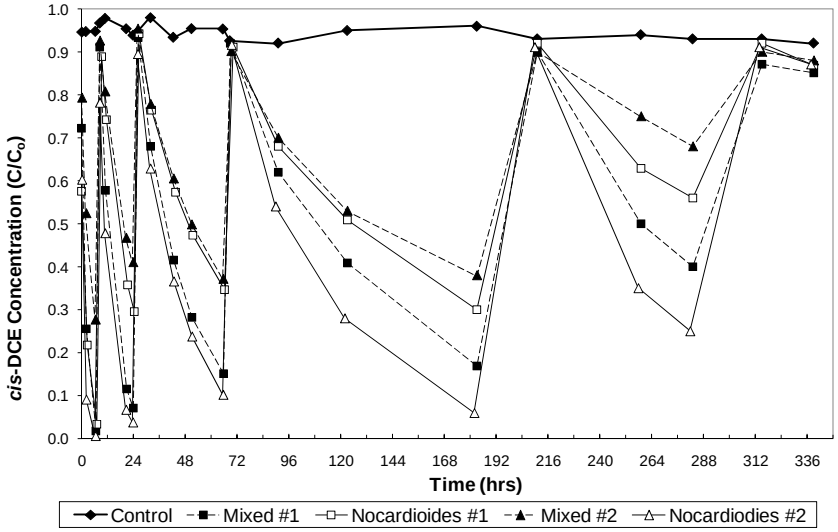

Figure 7. *cis*-DCE concentrations in the mixed ethenotrophic culture and *Nocardiodes* strain JS614 culture over time.

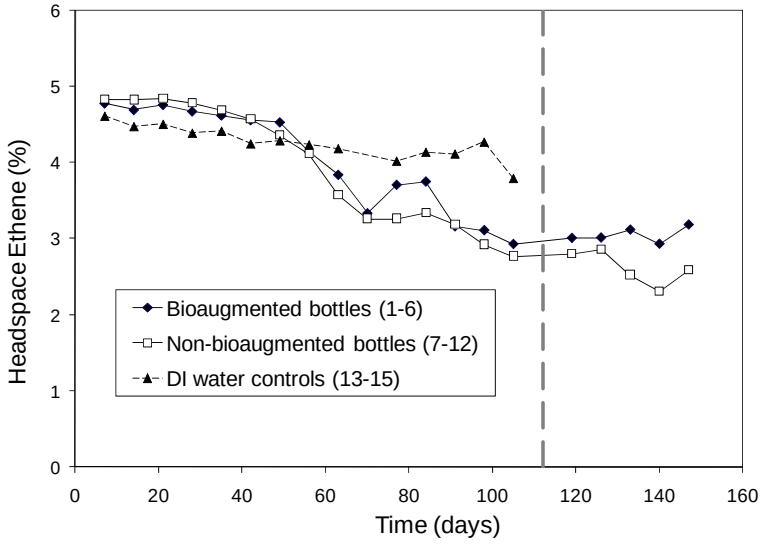

Figure 8. Average headspace ethene concentrations in the 12 microcosm bottles and 3 control bottles over time. On day 112 (vertical dashed line), bottles 1-12 were amended with nutrients, bottles 1-6 were bioaugmented, and control bottles 13-15 were inadvertently opened.

monitored over time. Figure 8 compares the average headspace ethene concentrations in the 12 microcosm bottles and the 3 control bottles over time. During days 1-49, the average headspace ethene concentrations in the microcosm bottles and in the control bottles were consistently between 4.4% and 4.8%, indicating negligible activity of ethenotrophic bacteria. However, between days 49 and 105, the average microcosm bottle headspace ethene concentrations slowly decreased from 4.4% to 2.9%, whereas the average control bottle headspace concentrations only decreased from 4.3% to 3.8%. Correspondingly, the average headspace CO_2 concentrations in

the microcosm bottles slowly increased from 0.23% to 0.55%, whereas the average headspace CO_2 concentrations in the controls did not increase significantly (data not shown). These results were consistent with the possibility that ethenotrophic bacteria were active in the microcosm bottles. Finally, no methane was detected, indicating that methanogens were not active.

3.2.3 Assessment of nutrient amendment and bioaugmentation on ethenotroph activity

Although the headspace ethene and CO_2 analyses did suggest that ethenotrophic bacteria may have been active in the microcosms, the disappearance of ethene was very slow. Therefore, on day 112, all the microcosm bottles were amended with 5 mL of mineral salts media (MSM) to supply nutrients (N and P), and bottles 1-6 were bioaugmented with 250 μL of the DCE-degrading mixed ethenotrophic culture and 250 μL of the pure culture of *Nocardioides* strain JS614. Surprisingly, the average headspace ethene concentrations in the bioaugmented bottles (1-6) did not decrease at all between day 105 (before bioaugmentation) and day 147 (35 days after bioaugmentation), whereas the average headspace ethene concentrations in the non-bioaugmented bottles did decrease slightly from 2.77% on day 105 to 2.59% on day 147. These results suggested that, although the mixed ethenotrophic culture and pure culture of *Nocardioides* strain JS614 both grew readily in MSM, the cultures did not acclimate readily to the EAFB aquifer sediments.

4. CONCLUSIONS

The studies with the enriched mixed ethenotrophic culture and the pure *Nocardioides* strain JS614 culture demonstrated that both were able to rapidly degrade *cis*-DCE when grown in a mineral salts medium. The *cis*-DCE degradation rates decreased significantly over time, indicating that neither culture could utilize *cis*-DCE as a growth substrate, and that the degradation occurred via cometabolism. The *cis*-DCE transformation yields were determined to be 0.128 ± 0.042 and 0.156 ± 0.015 mg *cis*-DCE/mg ethene, respectively. However, these cultures did not appear to acclimate to the EAFB aquifer sediments readily.

The experimental results of the aquifer sediment column studies indicated that simply supplying ethene and air to the EAFB aquifer sediments alone did not produce conditions favorable for growth of DCE-degrading ethenotrophs. Moreover, amending the EAFB aquifer sediments with nutrients and bioaugmenting with the enriched and pure ethenotrophic cultures failed to produce conditions favorable for growth of DCE-degrading ethenotrophs. This may have been due to the presence of inhibitory substrates (e.g., metals, salts, or organic compounds), or the absence of requisite growth factors (e.g., micronutrients).

Biodegradation and pervaporation were competing removal mechanisms in the column reactors, such that lower biodegradation removals corresponded to higher pervaporation removals. Thus, it was not surprising that *cis*-DCE pervaporation was a more significant removal mechanism in this study than for TCE in a similar previous study (Ma et al., 2003). One possible explanation for the ethene-supplied test column not achieving higher *cis*-DCE removal rates than the control column (when both columns had the same gas flow per membrane fiber) was that high pH conditions (8.2 to 9.1) inhibited growth of *cis*-DCE degrading ethenotrophic bacteria. However, a 5% ethene in air headspace did not stimulate significant growth of ethenotrophs in

the same EAFB aquifer sediments during pH-controlled microcosm studies (over a 21-week period), even after nutrient addition and bioaugmentation with DCE-degrading ethenotrophic cultures.

Future research should focus on the causes for inhibited growth of ethenotrophs in the aquifer sediments, including the possibility of the presence of inhibitory substrates (e.g., metals, salts, or organic compounds) or the absence of requisite growth factors (e.g., micronutrients).

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